PATENT
Attorney Docket: **056159-5261**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| In re Application of: **John W. Chapman *et al.*** | ) | Confirmation No.: **6003** |
| Application No. **10/539,229** | ) | Group Art Unit: **1656** |
| Filed: **April 27, 2006** | ) | Examiner: **David J. Steadman** |
| For: **Preparation of Antifreeze Protein** | ) | |

**DECLARATION UNDER 37 C.F.R. § 1.132**

I, the undersigned, John W. Chapman, do hereby declare that:

1. I am a citizen of the United Kingdom of Great Britain and Northern Ireland, residing at Cipreslaan 65, 3053NB, Rotterdam, The Netherlands.

2. I have been awarded Doctor of Philosophy from the United Kingdom Council for National Academic Awards (CNAA). I did my post-doctorate training at the UK National Institute for Medical Research, Mill Hill, London, England.

3. I am an inventor of the subject application and I am currently the Science Leader, Microbiology at the Unilever Research Laboratory at Vlaardingen, The Netherlands. I have been employed by Unilever N.V. since June 1992. Good Humor-Breyers Ice Cream, the assignee of the present application, is a unit of Unilever N.V.

4. I am familiar with the specification and the pending claims of U.S. Patent Application 10/539,229. I have reviewed the Office Action, dated May 6, 2009. I have also read the cited references used to allege that the claimed invention is obvious, including the references of Ng (U.S. Published Patent Application 2002/0068325) and Gentzsch (FEBS Lett. 377: 128-130, 1995). I believe that the feature of the claimed invention to produce type III antifreeze proteins in a glycosylation deficient yeast is novel and unobvious over the cited references.

5. In addition to U.S. Patent Application 10/539,229, I am also an inventor of the cited International Published PCT Patent Application WO 97/02343. That reference describes my invention of discovering the type III antifreeze protein (AFP) and producing the type III AFP in yeast. This protein was functional when expressed in wild-type yeast.

6. The Office Action, dated May 6, 2009, alleges that it would be obvious to express a protein in a glycosylation-deficient strain of yeast because the reference of Ng suggests the use of a glycosylation-deficient strain of yeast to overcome misfolding and reduced function and protein stability in an expressed protein. According to the invention of Ng, "the quality control mechanism employed by yeast which returns misfolded proteins to the cystosol for degradation is manipulated so that these proteins are instead secreted" (Ng at paragraph [0014]). However, since the type III AFP was functional when expressed in wild-type yeast, one would have expected that it was folded properly. Furthermore, the glycosylated form was secreted in high levels suggesting that it was not subject to degradation by the quality control mechanism described by Ng. Therefore, there was no reason to express it in a glycosylation-deficient strain of yeast which has a modification in the degradation pathway.

7. Prior to my present invention, it was unknown which enzymes, if any, would glycosylate type III AFP in *Saccharomyces cerevisae*. Further, the attached reference of Gentzsch (Glycobiology 7: 481-486, 1997) ("Gentzsch 2") discloses that there is no current way of predicting which enzymes will glycosylate a particular protein. Accordingly, one skilled in the art at the time of the invention would be unable to predict which enzymes should be deleted to alter the glycosylation of type III AFP.

8. Moreover, at the time of the present invention, it had been demonstrated that altering the glycosylation of the antifreeze had no effect on the stability and function of rye grass AFP. For example, in the attached reference of Pudney (Archives of Biochemistry and Biophysics 410: 238-245, 2003) at page 14, left column states that "glycosylation of the [AFP]...plays no role in the interaction with ice" suggesting that glycosylation is not important in maintaining stability and activity of AFP.

9. Further, at the time of my present invention, it was unknown what effect, if any, glycosylation would have on the type III AFP. Despite the teachings of the reference of Ng, it appeared that at the time of the present invention, modulating the glycosylation of a protein could have varied and unpredictable effects. For example, in contrast to the teachings of Ng, the attached reference of Sanders (Journal of Cell Biology 145: 1177-1188, 1999) describes that glycosylation is required for the stability and function of a protein expressed in yeast. Accordingly, one skilled in the art at the time of the present invention could not have predicted whether altering the glycosylation would have a positive or negative effect on the stability and function of the antifreeze protein. At the time of the present invention, one would not have expected that decreasing the glycosylation of a protein would have any effect on the stability and activity of type III AFP.

10. Therefore, in view of the state of the art at the time of the present invention, as is summarized by the attached three references, one skilled in the art would not know which enzymes or combination of enzymes to target to modulate glycosylation of the AFP. It was also reported in the art that glycosylation was not relevant to a function of the antifreeze protein. It was also known that glycosylation could be detrimental to expression of a protein. In view of this, the present invention of producing the antifreeze protein having increased activity in a pmt 1 or pmt 2 deficient yeast was a surprising and unpredictable result.

11. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 29th September 2009 By: _____

John Chapman, Ph.D.